Suppl Response Mail

Attorney's Docket No.. 10287-051001 / MGH 1470.0

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Michael J. Detmar et al.
Serial No. : 09/536,087
Filed : March 24, 2000
Title : THROMBOSPONDIN-2 AND USES THEREOF

Art Unit : 1642
Examiner : M. Wells

Commissioner for Patents
Washington, D.C. 20231

SUPPLEMENTAL RESPONSE

In response to the action mailed April 2, 2002, Applicants filed an amendment and response on October 1, 2002, which included a declaration of Dr. Michael Detmar under 37 C.F.R. §1.132. The figures accompanying the declaration were inadvertently omitted from the response as filed. Therefore, a copy of the declaration including the accompanying figures is submitted herewith in supplemental response. The declaration is otherwise identical to the one submitted with the response filed October 1, 2002.

Respectfully submitted,

Date: November 8, 2002

Louis Myers, Reg. No. 35,965

for: Louis Myers
Reg. No. 35,965

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CERTIFICATE OF MAILING BY FIRST CLASS MAIL

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DECLARATION UNDER 37 C.F.R. §1.132 OF DR. MICHAEL DETMAR

I, Michael Detmar, a citizen of Germany, residing in Arlington, MA, hereby declare as follows:

1. I am Associate Professor of Dermatology at Harvard Medical School and Associate Biologist at Massachusetts General Hospital. I received my M.D. degree from the University of Freiburg Medical School, Germany in 1984. After completion of my residency in dermatology at the Free University of Berlin, Germany in 1990, I held the position of Senior Dermatologist at the Dept. of Dermatology, Free University of Berlin until 1993. In 1993, I joined the faculty of the Dept. of Dermatology, Harvard Medical School, as a Visiting Assistant Professor of Pathology (1993-1996) and of Dermatology (1995-1997). In 1998, I joined the Dept. of Dermatology at Massachusetts General Hospital and was appointed Associate Professor of Dermatology, Harvard Medical School.

2. I am a co-inventor of the invention claimed in the above-identified patent application, and I have read and understand the contents of the present patent application.

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October 1, 2002
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Lenora H. Francis
Signature

LENORA H. FRANCIS
Typed or Printed Name of Person Signing Certificate

3. I have also been advised and understand that the Examiner has rejected claims 1, 13-23 and 53-74 of the above-referenced application which is directed to a method of treating a subject having a disorder characterized by unwanted cell proliferation. The method includes administering TSP-2 or a fragment of TSP-2 having the ability to inhibit endothelial cell migration. I further have been advised and understand that this rejection is based, in part, on the Examiner's assertion that "the specification does not give any guidance to which TSP-2 fragments will exhibit the biological activities as the claimed, or any guidance as to which regions of amino acid sequence are responsible for biological activity and thus, must be preserved so the molecule will function as claimed, or how to make and select for such molecules."

4. Contrary to the Examiner's statements, the specification provides sufficient guidance for a skilled artisan to practice the full scope of the claimed methods without undue experimentation. In particular, the TSP-2 sequence is known, and molecular biology techniques for making TSP-2 fragments are routine in the art. The specification describes the inhibition of tumor growth by TSP-2 in at least 2 different in vivo models: inhibition of squamous cell carcinoma cell grafts (A431 cells) and malignant melanoma cell grafts (MeWo cells) in mice (see, e.g., page 33, line 17 to page 34, line 19, and Figure 3A and 3B). The specification also clearly shows that TSP-2's anti-tumor growth activity correlates with its ability to inhibit endothelial cell migration, rather than with a direct effect on growth or proliferation of tumor cells (see, e.g., page 33, lines 6-16; and page 35, line 15, to page 36, line 12). Further, the specification provides at least 2 assays that can be used to identify TSP-2 fragments that have the required endothelial cell migration inhibitory activity and/or tumor inhibition activity. For example, an *in vitro* human dermal microvascular endothelial cell (HDMEC) migration assay is described in detail at pages 39-40; and a xenograft tumor growth assay combined with a vessel density assay in nude mice is described at page 33, line 17, to page 34, line 19. Indeed, the HDMEC migration assay alone was sufficient to identify a working example of an active TSP-2 fragment from only five fragments that were tested (47.6% inhibition by the peptide 7 (SEQ ID NO:10) compared to 54.2% inhibition by full length TSP2) (see page 39, line 24 to page 40, line 15). That one of

in vitro

merely five tested fragments gave a positive result is clearly indicative of the routine nature of the assay.

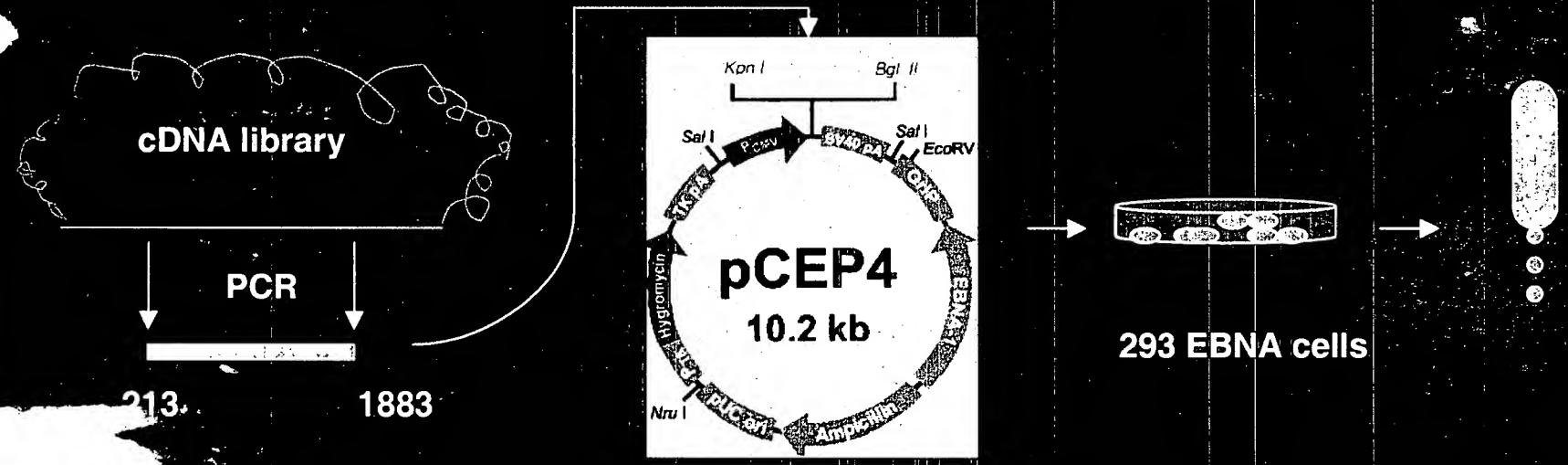
5. Moreover, using the methods described in the application, I have identified another fragment of TSP-2 that works in the claimed methods. I have made an N-terminal fragment of human TSP-2 (hTSP-2/NTF), encoded by nucleotides 213-1883 of SEQ ID NO:1 (see Figure 1 of declaration), containing the procollagen homology domain and 3 type-1 repeats of TSP-2. The fragment was effective to inhibit the migration of HDMEC cells as shown by using the same HDMEC assay as described in the specification (see Figure 2 of the declaration). The fragment was also effective to inhibit angiogenesis and growth of squamous cell carcinoma in vivo in mice using the same A431 xenotransplant assay as described in the specification (see Figures 3-5 of the declaration). This fragment includes the type I repeats of TSP-2, as taught at page 28, line 27 to page 29, line 5, of the specification, as an example of TSP-2 fragments that can be used in the claimed methods. This data clearly shows that one of ordinary skill in the art could perform the claimed methods using the guidance provided in the specification.

6. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title XVIII of the United States Code, and that such willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Michael Detmar, M.D.

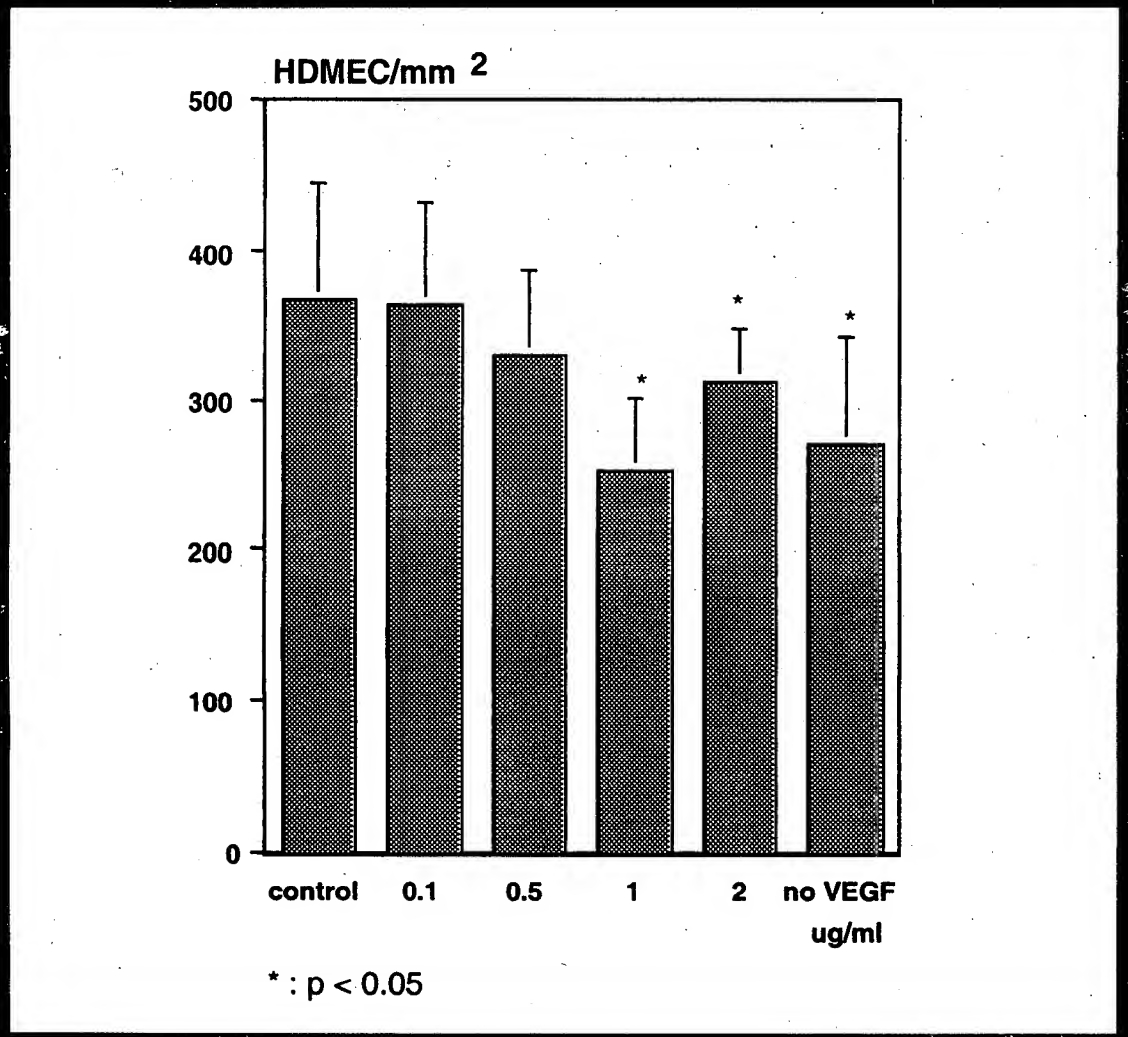
9/27/2002
Date

Preparation of recombinant human TSP-2 (N-terminal fragment)

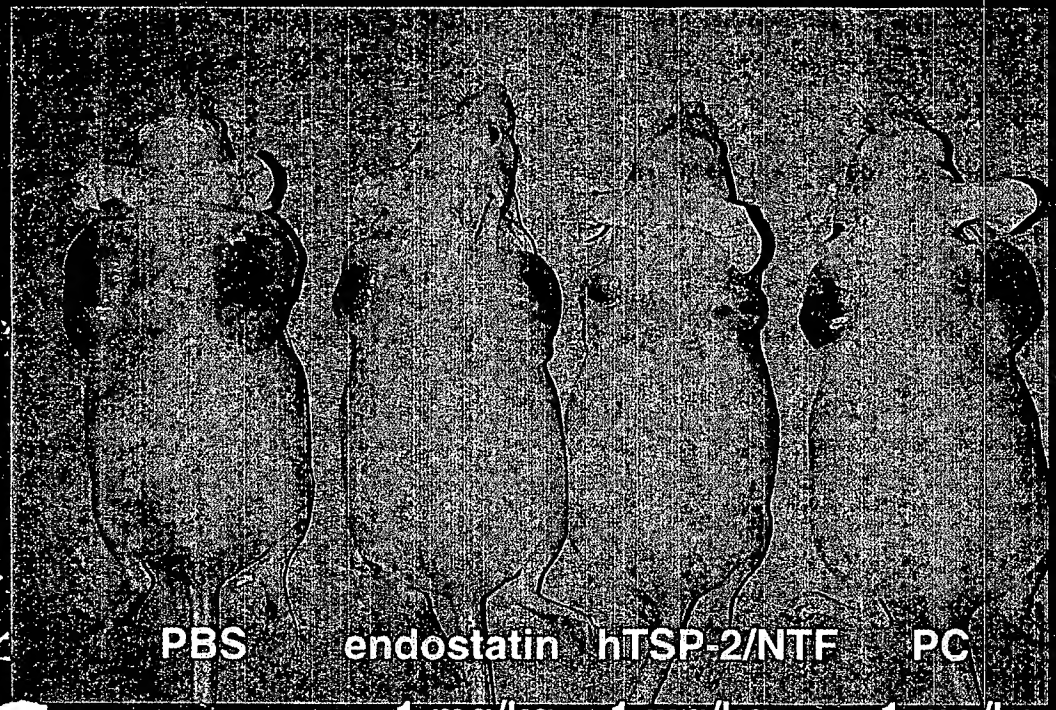


- PCR amplification of 1.6 kbp hTSP-2 DNA from human placenta cDNA library : amino terminal to type I repeats (nt 213-1883)
- Cloning into a modified pCEP 4 vector (*Kpn* I and *Bgl* II sites)
- Transfection into mammalian 293 EBNA cells
- Purification from culture supernatant with ammonium sulfate precipitation and gelatin- and heparin sepharose column chromatographies

hTSP-2/NTF inhibits the migration of human dermal microvascular endothelial cells (HDMEC).

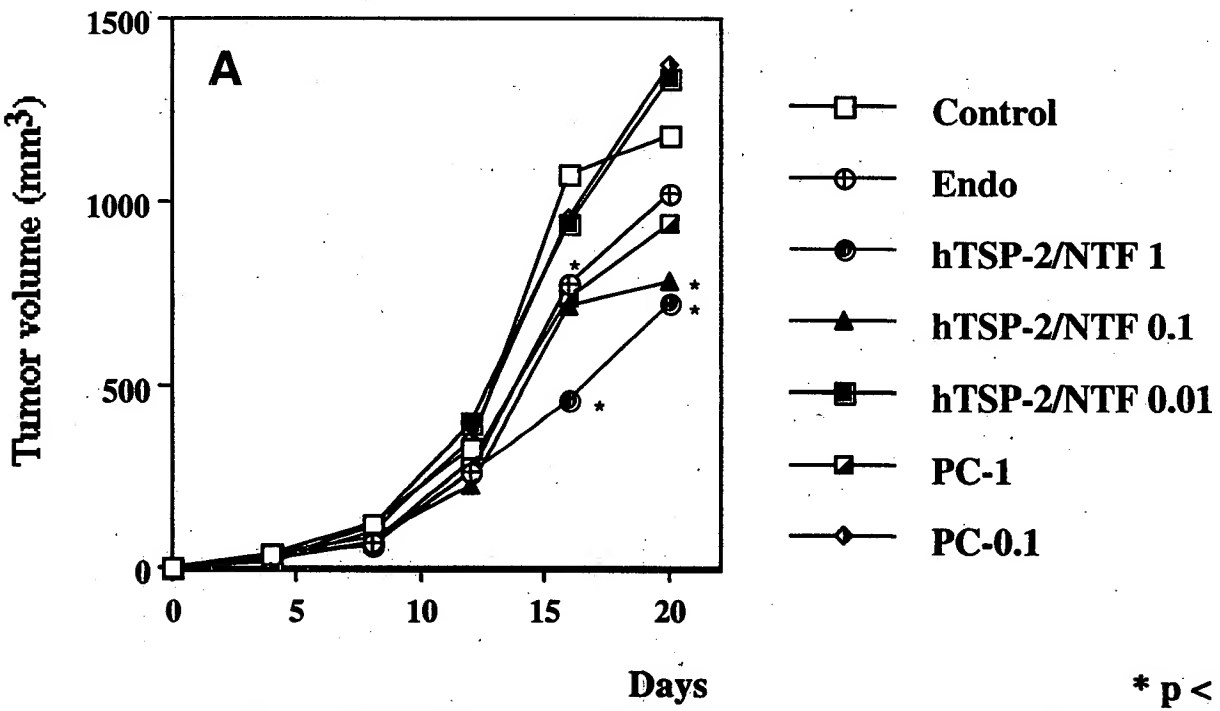


Systemic treatment with hTSP-2/NTF inhibits squamous cell carcinoma growth in mice (1).



- Nude mice: injected with 2 x 10^6 A431 cells intradermally.
- Treatment: daily i.p. injection with 1 or 0.1 mg/kg, beginning 2 days after tumor cell implantation.

A431 Tumor growth curve (1)



A431 Tumor growth curve (2)